3, lines 18-26, and page 5, lines 28-34 of the specification, as originally filed. Entry of the above amendments is respectfully requested.

ELECTION WITH TRAVERSE

The Examiner has required an election for examination purposes to one of the following inventions of Groups I-CXL.

Applicant hereby elects the invention of Group CXXXIV containing claims 39-41, 43, 45-47, and 51 drawn to a staphylokinase derivative having the code SY19 (S3C-MP5). This election is made with traverse on the grounds that the inventions of Groups I-CXL now contain a special technical feature in view of the above amendments to claims 31, 34, 35, 37, 38, and 46. Therefore, the invention of Groups I-CXL sufficiently relates to a single invention concept and should be examined in a single application.

The Examiner asserts that the inventions of Groups I-CXL lack the same or corresponding special technical feature and, therefore, do not relate to a single invention concept. Further, the Examiner asserts that the special technical feature of Groups I-CXL (i.e., staphylokinase derivatives with reduced immunogenicity) was known in the prior art before the priority date of the present application. However in view of the above amendments, the special technical feature that unites the inventions of Groups I-CXL include

staphylokinase derivatives that are typically modified with a polyethylene glycol and are characterized by a significantly reduced plasma clearance rendering them particularly suited for use by single intravenous bolus administration. This special technical feature (i.e.,"pegylated" staphylokinase variants) act as remarkably reduced doses in comparison to wild-type or non-pegylated, staphlokinase variants. (See page 3, lines 18-26 of the present specification). These "pegylated" staphylokinase derivatives were not known in the prior art before the priority date of the above application. The following enclosed references describing the "pegylated" staphylokinase derivatives (i.e., chemically modified with polyethylene glycol and characterized by a significantly reduced plasma clearance) are well after the priority date of the above application (i.e., Februry 1998).

Moons et al., "Toxicology studies with recombinant staphylokinase and with SY 161-P5, a polyethylene glycol-derivatized cysteine-substitution mutant." Toxicol Pathol. 2001 May-June;29(3):285-91.

Collen et al., "Polyethylene glycol-derivatized cysteine-substitution variants of recombinant staphylokinase for single-bolus treatment of acute myocardial infarction". Circulation, 2000 Oct 10;102(15): 1766-72.

Vanwetswinkel et al., "Pharmacokinetic and thrombolytic properties of cysteine-linked polyethylene glycol derivatives of staphylokinase". Blood. 2000 Feb 1;95(3): 936-42.

In addition, Applicant has not found any patent disclosures describing the "pegylated" staphylokinase derivatives before the priority date of the above patent application using the Delphion database.

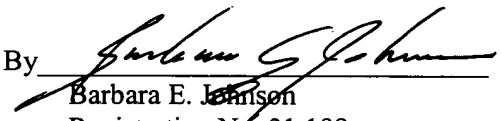
Therefore, the inventions of Groups I-CXL containing claims 30-51 now include the above special technical feature that links the groups of the invention as to form a single inventive concept.

In view of the foregoing amendments and remarks, it is respectfully requested that inventions of Groups I-CXL be examined in a single application.

If the transversal is unsuccessful, Applicant reserves the right to prosecute the non-elected claims at a later time by the way of a divisional application.

Respectfully submitted,

WEBB ZIESENHEIM LOGSDON
ORKIN & HANSON, P.C.

By 

Barbara E. Johnson
Registration No. 31,198
Attorney for Applicant
700 Koppers Building
436 Seventh Avenue
Pittsburgh, PA 15219-1818
Telephone: 412-471-8815
Facsimile: 412-471-4094

MARKED-UP AMENDED CLAIMS

31. (Once Amended) Staphylokinase derivatives having essentially the amino acid sequence as depicted in figure 1 in which one or more amino acids have been replaced by another amino acid thus reducing the reactivity with a panel of murine monoclonal antibodies provided that the other amino acid is not alanine, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

34. (Once Amended) Staphylokinase derivatives SakSTAR (K35X, G36X, E65X, K74X, E80X, D82X, K102X, E108X, K109X, K121X, K130X, K135X, K136X, +137X) having the amino acid sequence as depicted in figure 1 in which one or more of the amino acids Lys in position 35, Gly in position 36, Glu in position 65, Lys in position 74, Glu in position 80, Asp in position 82, Lys in position 102, Glu in position 108, Lys in position 109, Lys in position 121, Lys in position 130, Lys in position 135 and/or Lys in position 136 have been replaced with other amino acids provided that the other amino acid is not alanine and/or in which one amino acid has been added at the COOH-terminus, thus altering the immunogenicity after administration in patients, without markedly reducing the specific activity, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

35. (Once Amended) Staphylokinase derivatives listed in Tables 1, 3, 4, 5, 6, 7, 8, 13, 19, and 20, having the amino acid sequence as depicted in figure 1 in which the indicated amino acids have been replaced by other amino acids thus reducing the absorption of SakSTAR-specific antibodies from plasma of patients treated with staphylokinase, without reducing the specific activity, provided that at least one amino acid is replaced with an amino acid other than alanine, wherein the staphylokinase derivatives are chemically modified with polyethylene glycol and are characterized by a significantly reduced plasma clearance.

37. (Once Amended) SakSTAR (E65D, K74R, E80A, D82A, K130T, K135R) having the code SY19 which is chemically modified with polyethylene glycol and is characterized by a significantly reduced plasma clearance.

38. (Once Amended) SakSTAR (K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R) having the code SY161 which is chemically modified with polyethylene glycol and is characterized by a significantly reduced plasma clearance.

46. (Once Amended) Staphylokinase derivatives as claimed in claim 45, wherein selected amino acids in the NH_2-terminal region of 10 amino acids, are substituted with Cys, which is chemically modified with polyethylene glycol [with molecular weights up to 20 kDa, which derivatives] and is [are] characterized by a significantly reduced plasma clearance and maintained thrombolytic potency upon single intravenous bolus administration at a reduced dose.

1: Toxicol Pathol 2001 May-Jun;29 (3):285-91

Toxicology studies with recombinant staphylokinase and with SY 161-P5, a polyethylene glycol-derivatized cysteine-substitution mutant.

Moons L, Vanlinthout I, Roelants I, Moreadith R, Collen D, Rapold HJ.

Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, KU Leuven, Belgium.

SY 161-P5, a polyethylene glycol derivatized (PEGylated) mutant of the recombinant Staphylokinase (rSak) variant SakSTAR, exhibiting reduced antigenicity is in clinical development for treatment of acute myocardial infarction as a single bolus injection. A series of safety studies were performed in vivo as a routine toxicology program with SY 161-P5 (PEG-rSakSTAR) and with the recombinant Staphylokinase variant Sak42D (rSak42D). For both compounds, intravenous single bolus injections of up to 100-fold therapeutic equivalent, as well as repeated injections during 7 to 28 days revealed no significant pathological findings in mice, rats or hamsters. However, New Zealand white rabbits developed clinically silent, multifocal myocarditis following single or repeat doses of SY 161-P5 or of Sak42D. These findings were dose-independent and reversible. A similar species-specific cardiotoxic effect has previously been described for other proteolytic proteins, including the approved drugs Streptokinase and Acetylated Plasminogen Streptokinase Complex (APSAC). The large experience with these drugs, as well as the clinical data accumulated both with PEGylated and non-PEGylated rSak variants to date, do not indicate cardiotoxic hazards associated with the use of these drugs in humans.

PMID: 11442014 [PubMed - in process]

1: Circulation 2000 Oct 10;102(15):1766-72

Polyethylene glycol-derivatized cysteine-substitution variants of recombinant staphylokinase for single-bolus treatment of acute myocardial infarction.

Collen D, Sinnaeve P, Demarsin E, Moreau H, De Maeyer M, Jespers L, Laroche Y, Van de Werf F.

Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute for Biotechnology, KU Leuven, Belgium
desire.collen@med.kuleuven.ac.be

BACKGROUND: Thrombolytic therapy of acute myocardial infarction (AMI) is evolving toward bolus administration. Derivatization of proteins with polyethylene glycol (PEG) may reduce their clearance. METHODS AND RESULTS: A staphylokinase (SakSTAR) variant with 12 amino acid substitutions to reduce its antigenicity, SakSTAR (K35A, E65Q, K74R, E80A, D82A, T90A, E99D, T101S, E108A, K109A, K130T, K135R), and with Ser in position 3 mutated into Cys (code SY161), was derivatized with maleimide-PEG with M:(r) of 5,000 (P5), 10,000 (P10), or 20,000 (P20). The PEGylated variants recognized only one third of the antibodies elicited with wild-type SakSTAR in AMI patients. In experimental animals, plasma clearances were reduced 2. 5- to 5-fold with P5, 5- to 20-fold with P10, and 20-fold with P20, and bolus injection induced pulmonary plasma clot lysis at doses inversely related to their clearance. Intravenous bolus injection of 5 mg of the P5, P10, or P20 variants in AMI patients was associated with plasma half-lives (t (1/2alpha)) of 13, 30, and 120 minutes and clearances of 75, 43, and 8 mL/min, respectively, compared with 3 minutes and 360 mL/min for SakSTAR. Injection of 5 mg P5 variant restored TIMI-3 flow within 60 minutes in 14 of 18 AMI patients (78%, 95% CI 55% to 91%) and of 2.5 mg in 7 of 11 patients (63%, 95% CI 35% to 85%), both in the absence of fibrinogen degradation. The immunogenicity of the variants was significantly (P:<0.002) reduced. CONCLUSIONS: The staphylokinase variant SY161-P5, derivatized with one linear polyethylene glycol

molecule of M:(r) 5000, is a promising fibrin-selective agent for single-bolus coronary thrombolysis.

Publication Types:
- Clinical Trial
- Controlled Clinical Trial

PMID: 11023930 [PubMed - indexed for MEDLINE]

1: Blood 2000 Feb 1;95(3):936-42

Pharmacokinetic and thrombolytic properties of cysteine-linked polyethylene glycol derivatives of staphylokinase.

Vanwetswinkel S, Plaisance S, Zhi-Yong Z, Vanlinthout I, Brepoels K, Lasters I, Collen D, Jespers L.

Center for Transgene Technology and Gene Therapy, Flanders Interuniversity Institute of Biotechnology, Leuven, Belgium.

Recombinant staphylokinase (SakSTAR) variants obtained by site-directed substitution with cysteine, in the core (lysine 96 [Lys96], Lys102, Lys109, and/or Lys135) or the NH(2)-terminal region that is released during activation of SakSTAR (serine 2 [Ser2] and/or Ser3), were derivatized with thiol-specific (ortho-pyridyl-disulfide or maleimide) polyethylene glycol (PEG) molecules with molecular weights of 5,000 (P5), 10,000 (P10), or 20,000 (P20). The specific activities and thrombolytic potencies in human plasma were unaltered for most variants derivatized with PEG (PEGylates), but maleimide PEG derivatives had a better temperature stability profile. In hamsters, SakSTAR was cleared at 2.2 mL/min; variants with 1 P5 molecule were cleared 2-to 5-fold; variants with 2 P5 or 1 P10 molecules were cleared 10-to 30-fold; and variants with 1 P20 molecule were cleared 35-fold slower. A bolus injection induced dose-related lysis of a plasma clot, fibrin labeled with 125 iodine ((125)I-fibrin plasma clot), and injected into the jugular vein. A 50% clot lysis at 90 minutes required 110 microg/kg SakSTAR; 50 to 110 microg/kg of core-substitution derivatives with 1 P5; 25 microg/kg for NH(2)-terminal derivatives with 1 P5; 5 to 25 microg/kg with derivatives with 2 P5 or 1 P10; and 7 microg/kg with P20 derivatives. Core substitution with 1 or 2 P5 molecules did not significantly reduce the immunogenicity of SakSTAR in rabbits. Derivatization of staphylokinase with a single PEG molecule allows controllable reduction of the clearance while maintaining thrombolytic potency at a reduced dose. This indicates that mono-PEGylated staphylokinase variants may be used for single intravenous bolus injection.

PMID: 10648406 [PubMed - indexed for MEDLINE]